INTERLEUKIN-11 RECEPTOR

A NOVEL HAEMOPOIETIN RECEPTOR

The present invention relates generally to novel haemopoietin receptors, or components or parts thereof and to a method for cloning genetic sequences encoding same. More particularly, the subject invention is directed to recombinant or synthetic haemopoietin receptors or components or parts thereof. The receptor molecules or components or parts thereof and their genetic sequences of the present invention are useful in the development of a wide range of agonists, antagonists and therapeutics and diagnostic reagents based on ligand interaction with its receptor.

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

The proliferation, differentiation and function of a wide variety of cells are controlled by secreted regulators, known as cytokines. One such cytokine is interleukin (IL)-11 which is a functionally pleiotropic molecule (1,2), initially characterized by its ability to stimulate proliferation of the IL-6-dependent plasmacytoma cell line, T11 65 (3). Other biological actions of IL-11 include induction of multipotential haemopoietin progenitor cell proliferation (4,5,6), enhancement of megakaryocyte and platelet formation (7,8,9,10), stimulation of acute phase protein synthesis (11) and inhibition of adipocyte lipoprotein lipase activity (12, 13). The diverse and pleiotropic function of IL-11 makes it an important haemopoietin molecule to study, especially at the level of its interaction with its receptor.

The structure of the IL-11 receptor is not well known. It is known that neutralising

antibodies to gp130 inhibit IL-11-dependent proliferation of TF-1 cells (14) and, hence, it is likely that gp130 forms part of the receptor.

Members of the haemopoietin receptor family generally comprise α- and β-chains (15,16,17). However, until the advent of the present invention, there was no information on the existence of IL-11 receptor chains. In work leading up to the present invention, the inventors developed a cloning procedure for haemopoietin receptors which does not require prior knowledge of their ligands. The cloning procedure has been successful in cloning the IL-11 receptor α-chain permitting, for the first time, a detailed molecular analysis of the IL-11 receptor. The present invention provides, therefore, a generalized method for cloning haemopoietin receptors and in particular component chains thereof which provides a basis for developing a range of agonists, antagonists, therapeutic and diagnostic agents based on the IL-11 receptor.

Accordingly, one aspect of the present invention provides a genetic molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a haemopoietin receptor or a mutant, derivative, component, part, fragment, homologue, analogue or a peptide or polypeptide equivalent thereof wherein said receptor comprises an amino acid sequence set forth in SEQ ID NO: 1:

Trp-Ser-Xaa-Trp-Ser,

wherein Xaa is any amino acid.

More particularly, the present invention contemplates a genetic molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an IL-11 receptor or a mutant, derivative, component, part, fragment, homologue, analogue or a peptide or polypeptide equivalent thereof wherein said receptor comprises an amino acid sequence set forth in SEQ ID NO: 1:

Trp-Ser-Xaa-Trp-Ser,

wherein Xaa is any amino acid.

Another aspect of the present invention contemplates a method of identifying and/or cloning a genetic sequence encoding or complementary to a sequence encoding a

haemopoietin receptor and in particular an IL-11 receptor or a component or part thereof, said method comprising screening a source of genetic material with one or more degenerate oligonucleotides designed from the sequence of amino acids comprising the sequence set forth in SEQ ID NO: 1:

Trp-Ser-Xaa-Trp-Ser

wherein Xaa is any amino acid residue.

The sequence defined in SEQ ID NO: 1 has been identified in both α and β chains of haemopoietin receptors and in particular IL-11 receptor. Accordingly, the method of the present invention is applicable to the cloning of genetic sequences encoding an α-chain, a β-chain or a combination of both α- and β-chains such as in a full length receptor.

Preferably, the genetic molecule is of mammalian origin such as but not limited to humans, livestock animals (e.g. sheep, cows, pigs, goats, horses), laboratory test animals (e.g. mice, rats, guinea pigs), companion animals (e.g. cats, dogs) or captive wild animals. Most preferred origins are from humans and murine species (e.g. mice). The source of genetic material may be a genomic library or a cDNA library obtained from mRNA from a particular cell type such as would not limit to liver cells, bone marrow cells, placenta cells and heptatoma cells. A cDNA library is preferred and may also be an expression library. Furthermore, for the generation of mutants the cDNA library may be prepared by high error rate polymerase chain reaction (PCR) resulting in a mutant library.

The term "screening" includes any convenient means to identify target clones. For example, colony hybridization may be employed with oligonucleotide probes or if an expression library is prepared, screening may be, for example, enzyme activity or antibody interactivity. Terms such as "components", "parts" or "fragments" include separately an α-chain and a β-chain or parts thereof. Preferably, the "components", "parts" and "fragments" are functional and more preferably a functional α- or β-chain.

The genetic molecule may be single or double stranded, linear or closed circle DNA (e.g. genomic DNA), cDNA or mRNA or combinations thereof. The genetic molecule

may also include a vector such as an expression vector component to facilitate expression of the IL-11 receptor genetic sequence.

In a particular aspect, the genetic sequence encodes the α-chain of IL-11 receptor and in one preferred embodiment is murine IL-11 receptor α-chain encoded by a nucleotide sequence as set forth in SEQ ID NO: 2 or comprises an amino acid sequence as set forth in SEQ ID NO: 3, or comprises a part, derivative, fragment, portion, derivative, homologue, analogue or peptide equivalent thereof. In an alternative preferred embodiment, the genetic sequences encodes the α-chain of human IL-11 receptor and comprises the nucleotide sequence as set forth in SEQ ID NO: 4 or an amino acid sequence as set forth in SEQ ID NO: 5 or comprises a part, derivative, fragment, portion, derivative, homologue, analogue or peptide or polypeptide equivalent thereof. Accordingly, the genetic sequence may include a molecule capable of encoding a full length IL-11 receptor or a fragmented portion thereof such as an α-chain or a β-chain whether functional or not or may correspond to a portion thereof characterised by the amino acid sequence Trp-Ser-Xaa-Trp-Ser wherein Xaa is any amino acid residue. Additionally, the genetic sequence or part thereof may act as an antisense molecule or molecules to mRNA encoding the α- or β-chain of the IL-11 receptor. Such antisense molecules may be useful in genetic therapy or in the rational design of agonistic or antagonistic agents.

In a related embodiment, there is provided a genetic sequence which encodes an IL-11 receptor or a component, part or fragment thereof wherein said genetic sequence comprises a sequence of nucleotides to which SEQ ID NO: 2 or 4 may hybridise under low stringency conditions. In a further related embodiment, the genetic sequence is defined by the ability of an oligonucleotide selected from the following list to hybridise thereto:

5′ (A/G)CTCCA(C/T)TC(A/G)CTCCA 3′ (SEQ ID NO: 6);

5′ (A/G)CTCCA(A/G)TC(A/G)CTCCA 3′ (SEQ ID NO: 7);

5′ (A/G)CTCCA(N)GC(C/T)CTCCA 3′ (SEQ ID NO: 8);

5′ (A/G)CTCCA(N)GG(A/G)CTCCA 3′ (SEQ ID NO: 9);

5′ (A/G)CTCCA(C/T)TT(A/G)CTCCA 3′ (SEQ ID NO: 10);

or a complement sequence thereof or a combination thereof.

The present invention extends to the oligonucleotide defined by one of SEQ ID NOS: 1 to 6 and/or to labelled forms thereof or oligonucleotide stabilized to reduce nuclease-mediated action thereto.

For the purposes of defining the level of stringency, reference can conveniently be made to Sambrook *et al* (26) which is herein incorporated by reference where the washing steps at pages 9.52-9.57 are considered high stringency. A low stringency is defined herein as being in 0.1-0.5% w/v SDS at 37-45 C for 2-3 hours. Depending on the source and concentration of nucleic acid involved in the hybridisation, alternative conditions of stringency may be employed such as medium stringent conditions which are considered herein to be 0.25-0.5% w/v SDS at ≥ 45 C for 2-3 hours or high stringent conditions as disclosed by Sambrook *et al* (26).

The present invention is particularly useful for the cloning of haemopoietin receptor α- or β-chains, as exemplified by the cloning of the IL-11 receptor α-chain (IL-11rα). This is done, however, with the understanding that the present invention extends to a method for cloning all haemopoietin receptors including their α- or β-chains. Reference in the Examples to an α-chain is considered shorthand notiation to the entire receptor or various parts thereof, including the α- or β-chain.

In a further embodiment, the genetic sequence is fused to a heterologous genetic sequence to thereby encode a fusion molecule with, for example, glutathione-S-transferase, a receptor or subunit thereof for IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, erythropoietin, thrombopoietin, growth hormone, prolactin, CNTF, G-CSF, GM-CSF, gp130, or the p40 subunit of IL-12.

The genetic molecule may be single or double stranded, linear or closed circle DNA (e.g. genomic DNA), cDNA or mRNA or combinations thereof such as in the form of DNA:RNA hybrids. The genetic molecule may also include a vector such as an expression vector component to facilitate expression of the IL-11 receptor or its

components or parts. In a preferred embodiment, the genetic sequence encodes the α-chain of IL-11 having an amino acid sequence set forth in SEQ ID NO: 3 (murine) or SEQ ID NO: 5 (human) or comprises a part, derivative, fragment, portion, component, homologue or analogue of all or a portion thereof. Most preferably, the genetic sequence comprises a nucleotide sequence as set forth in SEQ ID NO: 2 (murine) or SEQ ID NO: 4 (human) or comprises a part, derivative, fragment, portion, component, homologue or analogue of all or part thereof.

The present invention further contemplates a kit useful for cloning a member of the haemopoietin receptor family or a component or part thereof, said kit comprising in compartmental form a first compartment adapted to contain at least one species of oligonucleotides having a nucleotide sequence based on the amino acid sequence SEQ ID NO: 1:

Trp-Ser-Xaa-Trp-Ser

wherein Xaa is any amino acid residue, said kit further optionally comprising one or more other compartments adapted to contain one or more other species of oligonucleotide based on SEQ ID NO: 1 and/or reagents required for a hybridisation assay for haemopoietin receptor genetic sequences. The kit may also be packaged for same with instructions for use. Preferred oligonucleotides include but are not limited to SEQ ID NO: 6 to 10.

Yet another aspect of the present invention is directed to a recombinant polypeptide comprising a sequence of amino acids corresponding to all or part of a mammalian IL-11 receptor α-chain. Preferably, the mammal is a human or a murine species such as a mouse. The polypeptide may correspond to a full length α-chain or may be a functional part, fragment or derivative thereof or may be a part, fragment or derivative having agonistic or antagonistic properties. In a preferred embodiment the polypeptide comprises an amino acid sequence as substantially set forth in SEQ ID NO: 3 (murine) or SEQ ID NO: 5 (human) or having at least about 40%, more preferably at least about 50%, still more preferably at least about 65%, even still more preferably at least about

75-80% and yet even more preferably at least about 90-95% or greater similarity to the sequence set forth in SEQ ID NO: 3 or SEQ ID NO: 5.

The polypeptide may have additional amino acid sequences fused thereto including GST, another cytokine, a receptor component or gp130. It may be glycosylated or unglycosylated depending on the cell used to produce same. Accordingly, the polypeptide may be produced in a prokaryotic cell (e.g. *E. coli* or *Bacilli* sp) or in a eukaryotic cell (e.g. mammalian cells such as BA/F3 cells [18] yeast cells, insect cells).

Mutants and derivatives of the recombinant polypeptide haemopoietin receptor properties include amino acid substitutions, deletions and/or additions. Furthermore, amino acids may be replaced by other amino acids having like properties, such as hydrophobicity, hydrophilicity, electronegativity, bulky side chains, interactive and/or functional groups and so on.

Amino acid substitutions are typically of single residues; insertions usually will be of the order of about 1-10 amino acid residues; and deletions will range from about 1-20 residues. Deletions or insertions preferably are made in adjacent pairs, i.e: a deletion of 2 residues or insertion of 2 residues.

The amino acid variants referred to above may readily be made using peptide synthetic techniques well known in the art, such as solid phase peptide synthesis and the like, or by recombinant DNA manipulations. Techniques for making substitution mutations at predetermined sites in DNA having known sequence are well known, for example through M13 mutagenesis. The manipulation of DNA sequences to produce variant proteins which manifest as substitutional, insertional or deletional variants are well known in the art.

Other examples of recombinant or synthetic mutants and derivatives of the recombinant haemopoietin receptor polypeptide of the present invention include single or multiple substitutions, deletions and/or additions to any molecule associated with the ligand such as carbohydrates, lipids and/or proteins or polypeptides. Naturally occurring or altered

glycosylated forms of the subject ligand are particularly contemplated by the present invention.

Amino acid alterations to the subject polypeptide contemplated herein include insertions such as amino acid and/or carboxyl terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Generally, insertions within the amino acid sequence will be smaller than amino or carboxyl terminal fusions, of the order of say 1 to 4 residues. Insertional amino acid sequence variants are those in which one or more amino acid residues are introduced into a predetermined site in the protein. Deletional variants are characterised by the removal of one or more amino acids from the sequence. Substitutional variants are those in which at least one residue in the sequence has been removed and a different residue inserted in its place. Such substitutions may be made in accordance with Table 1:

TABLE 1

Original Residue	Exemplary Substitutions
Ala	Ser
Arg	Lys
Asn	Gln; His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Pro
His	Asn; Gln
Ile	Leu; Val
Leu	Ile; Val
Lys	Arg; Gln; Glu
Met	Leu; Ile
Phe	Met; Leu; Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

The terms "analogues" and "derivatives" also extend to any functional chemical equivalent of the ligand characterised by its increased stability and/or efficacy *in vivo* or *in vitro*. The terms "analogue" and "derivatives" further extend to any amino acid derivative of the ligand as described above.

Analogues of the haemopoietin polypeptide receptor contemplated herein include, but are not limited to, modifications to side chains, incorporation of unnatural amino acids and/or derivatising the molecule and the use of crosslinkers and other methods which impose conformational constraints on the peptides or their analogues. Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with $NaBH_4$; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5'-phosphate followed by reduction with $NaBH_4$.

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3- butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbomoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during protein synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids.

Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with n = 1 to n = 6, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides could be conformationally constrained by, for example, incorporation of C_α and N_α-methylamino acids, introduction of double bonds between C_α and C_β atoms of amino acids and the formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

The present invention, therefore, extends to peptides or polypeptides and amino acid and/or chemical analogues thereof having the identifying characteristics of the α-chain of IL-11 receptor.

Accordingly, reference herein to the α-chain of the IL-11 receptor or a polypeptide having IL-11 α-chain properties includes the naturally occurring molecule, recombinant, synthetic and analogue forms thereof and to any mutants, derivatives and human and non-human homologues thereof including amino acid and glycosylation variants.

The availability of recombinant IL-11 receptor α-chain and genetic sequences encoding same permits for the first time the development of a range of agonists, antagonists, therapeutics and diagnostics to treat a variety of conditions involving a deficiency of IL-11, an excess amount of IL-11 or aberrant effects of normal endogenous IL-11 levels. Accordingly, the present invention extends to these agonists, antagonists, therapeutics and diagnostics and to compositions, pharmaceutical compositions and agents comprising one or more of same.

The present invention further described by the following non-limiting Figures and/or Examples.

In the Figures:

Figure 1 is a representation of the nucleotide sequence, predicted amino acid sequence and cDNA structure of the IL-11 receptor α-chain (IL-Nr1); (A) Structure of the IL-11rα cDNA, showing the 5′ and 3′ untranslated regions (solid line) and the coding region containing the predicted signal sequence (▨), the mature extracellular domain (□), transmembrane domain (▥) and cytoplasmic domain (▩). The size and extent of each of the IL-11rα cDNA clones that were sequenced completely are shown below. (B). The nucleotide and predicted amino acid sequence of IL-11rα. The untranslated region is shown in lower case and the coding region in upper case. The conventional one letter code for amino acids is employed throughout. The two potential asparagine-linked glycosylation sites (NXS/T) are shown underlined and in bold type. The potential signal sequence and the transmembrane domain are highlighted by bars between the nucleotide and amino acid sequence. The haemopoietin domain (D200) is boxed, and the broken line separates the two SD100 domains that comprise the D200 domain. A consensus polyadenylation signal in the 3′-untranslated region is shown in bold type.

Figure 2 is a comparison of Nr1 with other members of the haemopoietin receptor family; Amino acid sequence alignment of murine Nr1, the murine IL-6 receptor α-chain, the human CNTF receptor α-chain, the p40 subunit of human IL-12 and the murine GM-CSF receptor α-chain. Alignments were carried out by eye.

Figure 3 is a photographic representation of reverse transcriptase polymerase chain analyses of Nr1 mRNA; Cytoplasmic RNA was prepared from the following sources; lane 2, 3T3-L1 cells; lane 3, BAd cells; lane 4, UMR-106 cells; lane 5, PC13 cells; lane 6, NFS-60 cells; lane 7, FDCP-1 cells; lane 8 32D cells; lane 9, D35 cells; lane 10, M1 cells; lane 11, J774 cells; lane 12 WEHI-3B D-cells; lane 13, human bone marrow; lane 14, mouse bone marrow; lane 15, mouse spleen; lane 16, mouse thymus; lane 17, mouse ovary; lane 18, mouse uterus; lane 19, mouse testis; lane 20, mouse epididymus; lane 21, mouse brain; lane 22, mouse heart; lane 23, mouse kidney; lane 24 mouse thigh muscle; lane 25; mouse liver and lane 26, mouse salivary gland. 1 μg of each RNA sample and a control containing no RNA (lane 1) was subject to reverse transcription, with an identical reaction performed in the absence of reverse transcriptase. 5% of first strand cDNA reaction was subjected to PCR with primers specific for Nr1 (upper panel) or the control GAPDH (lower panel). PCR products were resolved on a 1.0% w/v agarose gel, transferred to nitrocellulose and hybridised with internal oligonucleotides specific to GAPDH or Nr1.

Figure 4 is a graphical representation of scatchard analyses of saturation isotherms of IL-11 binding to various cell lines; (A) parental Ba/F3 cells (●), Ba/F3 cells expressing Nr1 (○), Ba/F3 cells expressing Nr1 and the LIF receptor (■), (B) Ba/F3 cells expressing the LIF receptor and gp130 (●), Ba/F3 cells expressing Nr1 and gp130 (■), Ba/F3 cells expressing the Nr1, LIF receptor and gp130 (○), (C) parental M1 cells (●), M1 cells expressing Nr1 (○), and (D) 3T3-L1 cells (■) were incubated with various concentrations of labelled IL-11 in the presence of absence of a 10-100-fold excess of unlabelled IL-11. After 18 hours incubation on ice, bound and free IL-11 were separated by centrifugation through foetal calf serum. Bound and free ^{125}I-IL-11 was quantitated in a γ-counter and the data was depicted as a Scatchard transformation. In

each case data were normalised for cell number and shown as binding to 10^6 cells.

Figure 5 shows the molecular specificity of IL-11 binding to various cell lines: Ba/F3 cells expressing the designated receptors were incubated in 100 µl of medium containing 60,000 cpm (Ba/F3 Nr1) or 6,000 cpm of ^{125}I-IL-11 (Ba/F3 Nr1/gp130 and Ba/F3 Nr1/gp130/LIF receptor), in the presence or absence of 20 ng IL-11 or 200 ng of IL-6, LIF, OSM or IL-3. After 18 hours incubation on ice, bound and free IL-11 were separated by centrifugation through foetal calf serum. Bound and free ^{125}I-IL-11 were quantitated in a γ-counter and the amount of binding was expressed as a percentage of that observed in the absence of competitor.

Figure 6 shows differentiations of M1 cells expressing Nr1 in response to IL-11; 300 parental M1 cells (left panel) or M1 cells expressing Nr1 (right panel) were cultured in 1 ml of semi-solid agar with the designated concentration of LIF (◯) or IL-11(●). After 7 days, the proportion of colonies containing differentiated cells were determined.

Figure 7 shows factor dependent proliferation of Ba/F3 cells expressing various combinations of Nr1, gp130 and the LIF receptor; Parental Ba/F3 cells, Ba/F3 cells expressing Nr1, Ba/F3 cells expressing the Nr1 and the LIF receptor, Ba/F3 cells expressing LIF receptor and gp130, Ba/F3 cells expressing Nr1 and gp130 and Ba/F3 cells expressing Nr1, the LIF receptor and gp130 were incubated at 200 cells per well in a volume of 15µl, with the designated concentrations of IL-11 (●), IL-3(□) or LIF(◯), or with 3 µg/ml IL-6 and 500 ng/ml soluble IL-6 receptor α-chain (▲). After 48 hours the numbers of viable cells were counted.

Figure 8 is a representation of the composite nucleotide sequence and the predicted amino acid sequence of the human IL-11 receptor α chain. The predicted amino acid sequence is displayed using the conventional single letter code. The asterisk represents the termination codon. The four conserved cysteine residues, the WSTWS motif and the potential asparagine-linked glycosylation sites (NXS/T) are shown in bold type and underlined. The potential signal sequence and the transmembrane region is displayed by thin underline and double underline, respectively. A consensus poly-adenylation

signal is shown in lower case and bold type. The boxed region represents the 200 amino acid hemopoietin domain (D200) and is composed of two 100 amino acid subdomains (SD100) as marked by the broken line. The two arrows indicate the position of intronic sequences present in some of the cDNA clones.

Figure 9 is a representation of a comparison of the predicted amino acid sequence of the human (H) and the murine (M) IL-11 receptor α chain. The asterisk symbol indicates identity. The hatch (#) marks represent gaps introduced to improve the alignment.

Figure 10 is a photographic representation of a Southern blot demonstrating cross-species hybridisation of (A) murine IL-11 receptor α chain cDNA probe (445 bp *Sph I/Sac* I fragment) and (B) of human IL-11 receptor α chain cDNA probe (560 bp *Pst I/Xba* I fragment from clone #17.1) to human (H) and to murine (M) genomic DNA digested with Hind III. Nylon membrane processed under conditions of high stringency (0.2 X SSC, 0.1% w/v SDS, 65°C). Exposure was for 16 hours at -70°C using intensifying screens.

Figure 11 is a diagrammatic representation of structure of the human IL-11rα cDNA, displaying the 5′ and 3′ untranslated region (solid line) and the coding region containing the signal sequence (▥), the extracellular domain (□), the transmembrane region (▤), the cytoplasmic portion (■) and the poly A tail (AAAA). The approximate position of the conserved cysteine residues (C) and the WSTWS motif is indicated. The size and extent of the four cDNA clones selected for analysis is shown below. The approximate positions of the introns is indicated (V) as is their size in bp. The length of the clones is depicted without the introns. The composite cDNA was obtained from clines #9.1 and #17.1 by ligation at the indicated Pst I site (arrow) and used for expression studies.

Figure 12 is a diagrammatic representation of scatchard analyses of saturation isotherms of human IL-11 binding to M1 cells manipuated to express human IL-11rα ( ), M1 cells expressing the murine IL-11rα( ) and parental M1 cells (◯). Cells were incubated with various concentrations of labelled IL-11 in the presence of 10-100-fold excess of

unlabelled IL-11. After 18 hours incubation on ice, bound and free IL-11 were separated by centrifugation through FCS. Bound and free labelled IL-11 was quantitated n a γ counter and the data was depicted as a Scatchard transformation. In each case data were normalised for cell number and shown as binding to 10^6 cells. The amount of non-specific binding was between 0.1 and 1% of the total labelled IL-11 added. High-affinity binding was seen for M1 cells expressing human IL-11rα (K_d=250 pM) and urine IL-11rα (K_d=275 pM). Parental M1 cells did not display any specific binding.

Figure 13 is a photographic representation showing morphology of parental M1 cells and M1 cells manipulated to express the human IL-11 receptor α chain (M1/hIL-11rα) and in response to human IL-11 (1000 U/ml) and murine LIF (1000 U/ml). Cell morphology was examined after 5 days of incubation. Panels a, b and c show parental M1 cells with: normal saline (Panel a), LIF (Panel b) and IL-11 (Panel c). Panel d is representative of M1/hIL-11rα cells stimulated with IL-11 (X400).

Figure 14 is a graphical representation showing proliferation of parental Ba/F3 cells (▲), Ba/F3 cells manipulated to express the human IL-11 receptor α chain (Ba/F3+hIL-11rα) and Ba/F3 manipulated to express human IL-11 receptor α chain along with human gp130 (Ba/F3+hIL-11rα+gp130). Three clonal cell lines (Ba/F3+hIL-11rα) were established (represented by ●) that were unresponsive. Following the expression of the human gp130 molecule, all cell lines were IL-11 responsive (open symbols). Series dilutions of human IL-11 are shown. The results are means of triplicates from two experiments. All cells proliferated in IL-3.

The following single and three letter abbreviations for amino acid residues are used in the specification:

Amino Acid	Three-letter Abbreviation	One-letter Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any residue	Xaa	X

The following abbreviations are adopted in the subject specification:

IL-11:	Interleukin 11
IL-11r:	IL-11 receptor
IL-11rα:	IL-11 receptor α-chain
D:	Domain
SD:	Sub-domain
Nr1:	IL-11r

EXAMPLE 1

LIBRARY SCREENING

Commercial adult mouse liver cDNA libraries cloned into λgt10 and λZAP (Clonetech, CA, USA and Stratagene, CA, USA) were used to infect *Escherichia coli* of the strain LE392. Infected bacteria were grown on twenty 150 mm plates of agar, to give approximately 50,000 plaques per plate. Plaques were then transferred to duplicate 150 mm diameter nylon membranes (Colony/Plaque Screen ™, NEN Research Products, MA, USA), bacteria were lysed and the DNA was fixed by autoclaving at 100°C for 1 min with dry exhaust. The filters were rinsed twice in 0.1% w/v sodium dodecyl sulfate (SDS), 0.1 x SSC (SSC is 150 mM sodium chloride, 15 mM sodium citrate dihydrate) at room temperature and prehybridised overnight at 37°C in 6 x SSC containing 2 mg/ml bovine serum albumin, 2 mg/ml Ficoll, 2 mg/ml polyvinylpryrrolidone, 100 μM ATP, 10 μg/ml tRNA, 2 mM sodium pyrophosphate, 2mg/ml salmon sperm DNA, 0.1% NP-40 and 200 μg/ml sodium azide. The pre-hybridisation buffer was removed. An amount of 1.2 μg of the degenerate oligonucleotides for hybridisation (HYB1, HYB2 and HYB3; Table 1) were phosphorylated with T4 polynucleotide kinase using 960 μCi of γ^{32}P-ATP (Bresatec, S.A., Australia). Unincorporated ATP was separated from the labelled oligonucleotide using a pre-packed gel filtration column (NAP-5; Pharmacia, Uppsala, SWEDEN). Filters were hybridised overnight at 37°C in 80 ml of the prehybridisation buffer containing 0.1% w/v SDS, rather than NP40, and 10^6 - 10^7 cpm/ml of labelled oligonucleotide. Filters were briefly rinsed twice at room temperature in 6 x SSC, 0.1% v/v SDS, twice for 30 min at 45°C in a shaking waterbath

containing 1.5 l of the same buffer and then briefly in 6 x SSC at room temperature. Filters were then blotted dry and exposed to autoradiographic film at -70°C using intensifying screens, for 7-14 days prior to development.

Plaques that appeared positive on orientated duplicate filters were picked, eluted in 1 ml of 100 mM NaCl, 10 mM $MgCl_2$, 10 mM Tris.HCl pH7.4 containing 0.5% w/v gelatin and 0.5% v/v chloroform and stored at 4°C. After 2 days LE392 cells were infected with the eluate from the primary plugs and replated for the secondary screen. This process was repeated until hybridising plaques were pure.

EXAMPLE 2

ANALYSES OF POSITIVE PLAQUES

DNA was prepared from positive plaques using Promega Magic Lambda DNA columns (Promega Corporation, WI, USA) according to the manufacturer's instructions. An amount of 100 ng of DNA from each positive bacteriophage was sequenced using a fmol sequencing kit (Promega Corporation, WI, USA) with the ^{33}P-labelled oligonucleotide primers gt10*for*, gt10*rev* and either HYB1, HYB2 or HYB3. The products were resolved on a 6% w/v polyacrylamide gel and the sequence of each clone was analysed using the Blast database comparison programs and the translation function of the Wisconsin suite of programs.

The sequence of one clone (Nr1-AZ-36) contained motifs characteristic of the haemopoietin receptor family. Two oligonucleotides, #26 and #60 (nucleotides 946-970 and 1005-1034; Figure 1; Table 2), were designed from this sequence and used rescreen the primary filters from the original liver library and two other adult liver cDNA libraries. The initially isolated cDNA clone, Nr1-AZ-36, and four other cDNA clones (Nr1-30.2, 30.3, 30.4 and 30.17) were sequenced completely, on both strands, using the dideoxy method (18) with the Pharmacia T7 polymerase sequencing kit (Pharmacia, Uppsala, SWEDEN). The sequence of the new receptor was compared to the EMBL and Genbank database using the FASTA program. Alignments with known cytokine receptors were carried out by eye.

An alternative, quicker method for the analysis of positive plaques identified using degenerate oligonucleotides to the WSXWS motif.

Primary positive plaques are identified and picked.

5µl of primary plaque eluate was used in a polymerase chain reaction containing the following: 5µl 10x PCR buffer with Mg (Boehringer Mannheim), 1 µl 10 mM dATP, dCTP, dGTP and dTTP (Promega Corp), 2.5 µl of each primer at 100 µg/ml and 0.5µl of Taq polymerase (Boehringer Mannheim). The primers utilised were those WSXWS primers used in hybridisation in combination with primers specific to the λ-bacteriophage in which the library was cloned. PCR was carried in a Perkin Elmer 9600 machine using the following protocol: 96°C for 2 min, 25 cycles of 96°C for 30 sec, 55°C for 30 sec and 72°C for 2 min, 4°C indefinitely.

20µl of the PCR was electrophoresed on a 1% w/v agarose gel in TAE. Any products were isolated using GeneClean reagent and sequenced either using ^{33}P-labelled WSXWS primers with the fmol sequencing kit (Promega Corp) or unlabelled WSXWS primers and fluoresceinated dideoxy nucleotides with an automated sequencer. The sequence is then used to check for motifs common to receptors of the haemopoietin family.

TABLE 2

SEQUENCE OF OLIGONUCLEOTIDES

Oligonucleotide	Sequence	SEQ ID NO:
HYB1	5′ (A/G)CTCCA(C/T)TC(A/G)CTCCA 3′	6
HYB2	5′ (A/G)CTCCA(A/G)TC(A/G)CTCCA 3′	7
HYB3	5′ (A/G)CTCCA(N)GC(C/T)CTCCA 3′	8
#26	5′ TGGTCCACGGTGGAGCCCATTGGCT 3′	11
# 60	5′ CCACACGCGGTACGAGTCAGTGCCAGGGAC 3′	12
gt10*for*	5′ AGCAAGTTCAGCCTGGTTAAG 3′	13
gt10*rev*	5′ CTTATGAGTATTTCTTCCAGGGTA 3′	14
#495	5′ CCCTTCATTGACCTCAACTACATG 3′	15
#496	5′ CATGCCAGTGAGCTTCCCGTTCAG 3′	16
#449	5′ GGGTCCTCCAGGGGTCCAGTATGG 3′	17
#285	5′ GGAGGCCTCCAGAGGGT 3′	18
#489	5′ CTCCTGTACTTGGAGTCCAGG 3′	19
#741	5′ GGAAAGCTGTGGCGTGATGGCCGTGGGGCA 3′	20
30f1	5′ GGGCGGAGGCCGCTGGCGGGCG 3′	21
30r1	5′ TTATCAGCTGAAGTTCTCTGGGG 3′	22

EXAMPLE 3

REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION

First strand cDNA synthesis was performed on 1 µg of polyA+ cytoplasmic RNA. Reverse transcription was carried out at 42°C for 60 min in 20µl of 50 mM Tris.HCl pH8.3, 20 mM KCl, 10 mM $MgCl_2$, 5mM dithiothreitol, 1 mM of each dNTP, 20µg/ml oligo $(dT)_{15}$ and 12.5 units of AMV reverse transcriptase (Boehringer Mannheim GmbH, Mannheim, Germany). Control reactions were performed for each RNA sample under identical conditions except that reverse transcriptase was omitted from the reaction. The reverse transcription reaction mixture was diluted to 100µl with water and 5µl was used

for each PCR reaction. PCR reactions were carried out in 50µl of reaction buffer (Boehringer Mannheim GmbH, Mannheim, Germany) containing 200 µM of each dNTP, 1µM of each primer and 2.5 U of Taq polymerase (Boehringer Mannheim GmbH, Mannheim, Germany). The primers used for amplification of IL-11 receptor α-chain (Nr1) cDNA were, from homologY with other members of the haemopoietin receptor family, predicted to span at least one intron. These oligonucleotides were #449 and #285 (nucleotides 133-156 and 677-661; Figure 1, Table 2), while for amplification of GAPDH cDNA primers #495 and #496 were used (Table 2). PCR was performed for 30 cycles at 94°C for 2 min, at 60°C for 2 min and at 72°C for 3 min in a Perkin Elmer Cetus Thermal cycler (Perkin Elmer Cetus, CA, USA). An aliquot of the reaction mixture was electrophoresed on a 1.0% w/v agarose gel and DNA was transferred to a zetaprobe membrane. Southern blots were performed as described by Reed and Mann (19). Hybridisation was carried out with end-labelled oligonucleotides (#489 for the IL-11 receptor α-chain and #741 for GAPDH; Table 2).

EXAMPLE 4

EXPRESSION CONSTRUCTS

Nr1-30.3 was used in a PCR with primers 30f1 and 30r1 (Table 2) to generate a cDNA that contained little 5′ or 3′ untranslated region. The PCR product was cloned into the BstX I site of pEF-BOS (21) using BstX I adaptors (Invitrogen, CA, USA). The cDNA insert was sequenced on both strands. cDNAs encoding the human LIF receptor and mouse gp130 were also subcloned into pEF-BOS. Receptor cDNAs in pEF-BOS were linearized with Aat II prior to transfection. pBluescript derivatives containing cDNAs encoding the selectable markers puromycin transferase (pPGKpuropA) and neomycin transferase (pPGKneopA) transcribed from a PGK promoter and with the β-globin 3′-untranslated region were linearised with Sca I.

EXAMPLE 5

CELL TRANSFECTION

Cells were stably transfected by electroporation. Briefly, cells were washed twice in ice cold PBS and resuspended in PBS at 5 x 10^6 per ml. An amount of 4 x 10^6 cells was aliquoted into 0.4 mm electroporation cuvettes with 20μg of pEF-BOS with or without Nr1, gp130 or the LIF receptor cloned into the BstX I site and 2 μg of the selectable markers pPGKpuro or pPGKneo. DNA and cells were incubated for 10 minutes on ice and electroporated at 270 V and 960 μF in a Bio-Rad Gene Pulser (Bio-Rad Laboratories, CA, USA). The cells were mixed with 1 ml of culture medium, centrifuged through 3 ml of FCS and resuspended in 100 ml of culture medium. Cells were than aliquoted into four 24 well dishes. After two days, selection was commenced by the addition of geneticin to a concentration of 1.2 mg/ml, of puromycin to a concentration of 40 μg/ml for M1 cells and 5 μg/ml for Ba/F3 cells. After 10-14 days, clones of proliferating cells were transferred to flasks and, after expansion, tested for receptor expression.

EXAMPLE 6

CYTOKINES

Murine IL-3 and IL-11 were purchased from PeproTech (PeproTech, NJ, USA), human LIF and human OSM were produced using the pGEX system, essentially as described (25).

EXAMPLE 7

BIOLOGICAL ASSAYS

The proliferation of Ba/F3 cells in response to cytokines was measured in Lux60 microwell HL-A plate (Nunc Inc., IL, USA). Cells were washed three times in DME containing 20% v/v new born calf serum and resuspended at a concentration of 2 x 10^4 cells per ml in the same medium. Aliquots of 10 μl of the cell suspension were placed in the culture wells with 5μl of serial dilutions of purified recombinant IL-3, IL-11 or LIF, or IL-6 at 3 μg/ml and soluble IL-6 receptor α-chain at 500 ng/ml. After 2 days of incubation at 37°C in a fully humidified incubator containing 10% v/v CO_2 in air,

viable cells were counted using an inverted microscope.

In order to assay the differentiation of M1 cells in response to cytokines, 300 cells were cultured in 35 mm Petri dishes containing 1ml of DME supplemented with 20% v/v FCS, 0.3% w/v agar and 0.1 ml of serial dilutions of IL-6, IL-11, LIF or OSM. After 7 days culture at 37°C in a fully humidified atmosphere, containing 10% v/v CO_2 in air, colonies of M1 cells were counted and classified as differentiated if they contained dispersed cells or a corona of dispersed cells around a tightly packed centre.

EXAMPLE 8

BINDING STUDIES WITH IL-11

IL-11 was dissolved at a concentration of 100 µg/ml in 50 mM sodium phosphate, 150 mM NaCl (PBS), 0.02% v/v Tween 20 and 0.02% w/v sodium azide at pH 7.4. IL-11 was radio-iodinated according to the method of Bolton and Hunter (24). Briefly, 2 µg of IL-11 was incubated with 2 mCi of monoiodinated Bolton-Hunter reagent (New England Nuclear, MA, USA) at room temperature in 20 µl of 150 mM sodium borate pH 8.5. After two hours the reaction was quenched with 100µl of 1M glycine in the same buffer and the labelled protein was separated from unincorporated Bolton-Hunter reagent using a pre-packed Sephadex G-25 column (PD-10; Pharmacia, Uppsala, Sweden) equilibrated in PBS containing 0.02% v/v Tween 20 and 0.02% w/v sodium azide. Prior to use the ^{125}IL-11 was diluted 10-fold with 50 mM Tris HCl pH 7.5, containing 0.02% v/v Tween 20 and 0.02% w/v sodium azide and applied to a 250 µl column of CM-Sepharose CL-4B (Pharmacia, Uppsala, SWEDEN) equilibrated in the same buffer. The column was washed with 5 ml of equilibration buffer and eluted with sequential 5 ml aliquots of DME containing 10% v/v FCS. At this stage the ^{125}I was greater than 95% precipitable with cold trichloroacetic acid. The bindability of the ^{125}I-IL-11 preparation was assessed as previously described (21) and was approximately 80%. The specific radioactivity of the ^{125}I-IL-11 was approximately 130,000 cpm/ng and was determined by self-displacement analysis (22).

Binding studies were performed essentially as previously described (22). Briefly, 5 x 10^5 - 1.5 x 10^7 cells in 40 µl RPMI-1640 medium containing 20 mM Hepes pH 7.4 and

10% v/v foetal calf serum (RHF), were incubated overnight on ice, with between 5 x 10^3 and 2 x 10^6 cpm of ^{125}I-IL-11, with or without a 100-fold excess of unlabelled IL-11. In other experiments receptors were saturated with constant amount of ^{125}I-IL-11 and increasing amounts of unlabelled IL-11 or unlabelled IL-3, IL-6, LIF, OSM or G-CSF. Cell associated and free ^{125}I-IL-11 were separated by rapid centrifugation through 180µl of foetal calf serum and quantitated in a γ-counter.

EXAMPLE 9

CLONING CYTOKINE RECEPTORS ON THE BASIS OF SEQUENCE SIMILARITY

Members of the haemopoietin receptor family exhibit a relatively low level of sequence similarity. One of the features of receptors in this family is the five amino acid motif Trp-Ser-Xaa-Trp-Ser (WSXWS) (15, 16, 17). In an attempt to clone novel haemopoietin receptors, 10^6 plaques from an adult mouse liver cDNA library were screened with degenerate oligonucleotides corresponding to the WSXSW motif. λ-bacteriophage plaques that appeared positive on the duplicate primary filters were picked, eluted and isolated by two subsequent rounds of plaque enrichment. DNA from pure hybridising plaques was then sequenced.

The utility of this technique was demonstrated by the identification of several cDNAs encoding the murine LIF receptor, IL-7 receptor, gp130 and a novel sequence that appeared related to members of the haemopoietin receptor family which is termed herein "Nr1". The cDNA (Nr1-AZ-36) encoding this novel receptor was sequenced fully and although it contained a polyadenylation signal and an extensive poly-A tail, it was clearly truncated at the 5′ end (Figure 1).

EXAMPLE 10

ISOLATION OF FULL LENGTH Nr1 cDNA AND CHARACTERISATION OF THE NOVEL CYTOKINE RECEPTOR

To isolate a full length Nr1 cDNA, the original library and a second adult mouse liver cDNA library were screened with oligonucleotides (#26 and #60; Table 2) designed from the 5′ end of clone Nr1-AZ-36. Eight cDNA clones were isolated and four were

sequenced completely (Figure 1). Analyses of the cDNA sequences revealed an open reading frame of 1296 bp which encoded a protein of 432 amino acids in length. The predicted primary sequence included a potential hydrophobic leader sequence (residues 1-23), extracellular domain with two potential N-linked glycosylation sites (residues 24-367), transmembrane domain (residues 368-393) and short cytoplasmic tail (residues 394-432). The core molecular weight of the mature receptor has been initially estimated to be approximately 36,000 daltons.

The extracellular domain contained residues characteristic of a classical haemopoietin domain (D200; 15) (Figures 1 and 2), including proline residues preceding each 100 amino acid sub domain (SD100), four conserved cysteine residues, a series of polar and hydrophobic residues, and a WSXWS motif. The haemopoietin receptor domain of the new receptor was preceded by an 87 amino acid immunoglobulin-like domain and followed by 37 amino acids before the transmembrane domain. Regarding its overall structure and its primary sequence (Figure 2), the new receptor was most similar to the IL-6 receptor α-chain (24% amino acid identity), the CNTF receptor α-chain (22% amino acid identity) and the p40 subunit of IL-12 (16% amino acid identity).

EXAMPLE 11

EXPRESSION OF Nr1 mRNA

The distribution of Nr1 mRNA expression was analysed by Northern blot and reverse transcriptase polymerase chain reaction (RT-PCR). Among a survey of polyadenylated RNA from 15 primary tissue samples and 17 cell lines, only RNA from the pre-adipocyte cell line 3T3-L1, yielded a detectable hybridising band of approximately 2.0kb in length on a Northern blot. This compares to a length of approximately 1650 bp for the longest Nr1 cDNA isolated and suggests that this clone may not be complete at the 5' end.

The low abundance of the Nr1 mRNA suggested from Northern analyses prompted the use of RT-PCR as a more sensitive means of detection. All samples contained GAPDH mRNA as judged by RT-PCR (Figure 3), however only 3T3-L1 cells, the stromal line BAd, the embryonic carcinoma cell line PC13 and the factor dependent haemopoietin

cell lines FDCP-1 and D35 expressed Nr1 mRNA (Figure 3). A wide range of primary tissues were also positive (Figure 3) including the haemopoietin tissues bone marrow, spleen and thymus as well as the liver, brain, heart, kidney, muscle and salivary gland. In mRNA samples from several cell lines and tissues transcripts for Nr1 could not be detected. Such negative results need to be confirmed using a more quantitative approach to mRNA analysis. In control experiments, PCR was performed on mRNA that had not been subjected to reverse transcription. In none of these samples was a Nr1 product detected.

EXAMPLE 12

Nr1 IS A LOW AFFINITY RECEPTOR FOR IL-11 AND INTERACTS WITH gp130 TO GENERATE A HIGH AFFINITY IL-11 RECEPTOR

Given its sequence similarity with the IL-6 and CNTF receptor α-chains and its expression in 3T3-L1-cells, it was reasoned that Nr1 might be a receptor α-chain which interacts with gp130 and/or the LIF receptor to generate a high affinity receptor capable of signal transduction. Since no receptor α-chains, similar in structure to the IL-6 receptor α-chain, have been described for LIF, OSM and IL-11, these cytokines represent attractive candidates for the cognate ligand of Nr1.

To test whether LIF, OSM or IL-11 bound to the new receptor, the factor-dependent haemopoietin cell line Ba/F3 and the mouse leukaemic cell line M1 were stably transfected with the vector pEF-BOS containing the cDNA encoding Nr1. Parental M1 cells express the LIF receptor and gp130 and, therefore, bound ^{125}I-LIF and ^{125}I-OSM. Expression of Nr1 in M1 cells did not result in altered binding of either ^{125}I-LIF or ^{125}I-OSM. In contrast, Ba/F3 cells expressed neither the LIF receptor nor gp130 and no binding of ^{125}I-LIF and ^{125}I-OSM was observed on either parental Ba/F3 cells or cells expressing Nr1.

No binding of ^{125}I-IL-11 could be detected on parental M1 or Ba/F3 cells (Figure 4A & C). Strikingly, however, expression of Nr1 in each cell type resulted in the ability to bind ^{125}I-IL-11 which suggested that Nr1 might be the α-chain of the IL-11 receptor. Scatchard transformation of the saturation binding isotherms revealed that the affinity

of IL-11 for its receptor differed between the two cell types (Figure 4A versus 4C). Binding of ^{125}I-IL-11 to Ba/F3 cells expressing Nr1 was of very low affinity. The apparent equilibrium dissociation constant (K_D) for this interaction was estimated to be approximately 10 pM and cells expressed an average of between 2,000 and 8,000 receptors at their surface (Figure 4A). M1 cells transfected with a Nr1 cDNA expressed a similar number of IL-11 receptors (Figure 4C), however, the affinity of the interaction was higher (K_D=400-800 pM). The IL-11 receptors expressed on M1 cells transfected with Nr1 were similar in affinity to the receptors expressed naturally on 3T3-L1 cells (Figure 4D).

One explanation for the generation of low affinity or high affinity receptors according to the cell type in which Nr1 is expressed, is that Nr1 itself has an intrinsically low affinity for IL-11, but M1 cells express an excess of an additional receptor component required for the generation of a high affinity complex. Indirect evidence exists for the role of gp130 in IL-11 receptor signal transduction, since neutralising antibodies to gp130 inhibited IL-11 induced proliferation of TF-1 cells. In order to test this proposition directly, gp130 and/or the LIF receptor were expressed in parental Ba/F3 cells or in Ba/F3 cells expressing Nr1.

Parental Ba/F3 cells and Ba/F3 cells expressing gp130 and the LIF receptor, alone or in combination did not bind IL-11 (Figure 4A and B). Ba/F3 cells expressing Nr1 and the LIF receptor, bound IL-11 with a very low affinity that was indistinguishable from cells expressing IL-11 receptor α-chain alone (Figure 4A). In contrast, when gp130 and Nr1 were co-expressed in Ba/F3 cells, high affinity receptors for IL-11 were generated (Figure 4B). The affinity of these receptors was similar to that of receptors expressed by 3T3-L1 cells and M1 cells expressing IL-11 receptor α-chain (Figure 4B-D). Expression of the LIF receptor with Nr1 and gp130 did not increase the affinity of IL-11 binding (Figure 4B).

Nr1 appears to be a receptor that is specific for IL-11. The binding of ^{125}I-IL-11 to Ba/F3 cells expressing Nr1 was competed for by unlabelled IL-11, but not IL-6, LIF, OSM or IL-3 (Figure 5). A more complex situation exists in cells in which Nr1 is

expressed with gp130 and the LIF receptor. The binding of ^{125}I-IL-11 to Ba/F3 cells expressing Nr1 and gp130, was completed for by OSM and unlabelled IL-11 (Figure 5), while binding to Ba/F3 cells expressing Nr1, gp130 and the LIF receptor was competed for by LIF, as well as OSM and IL-11 (Figure 5).

EXAMPLE 13

CO-EXPRESSION OF IL-11 RECEPTOR α-CHAIN AND gp130 ALLOWS A PROLIFERATIVE AND DIFFERENTIATIVE RESPONSE TO IL-11

Many cytokines exert effects upon cell differentiation as well as cell division. In the absence of differentiative stimuli, colonies of parental leukaemic M1 cells are tightly packed and are composed of undifferentiated blast cells. In response to LIF, OSM and IL-6, but not IL-11, M1 colonies grown in semi-solid agar become dispersed because of the induction of macrophage differentiation (Figure 6A). In addition, LIF, OSM and IL-6 suppress the clonogenicity of M1 cells resulting in the development of reduced numbers of colonies. M1 cells expressing the IL-11 receptor α-chain exhibited a normal response to LIF, OSM and IL-6 but now differentiated into macrophages when stimulated by IL-11 (Figure 6B). As with LIF, IL-6 and OSM, fewer colonies were produced by M1 cells expressing Nr1 in the presence of IL-11 than in control cultures and these colonies contained fewer cells.

The IL-3-dependent haemopoietin cell line Ba/F3 has been used to study the capacity of a variety of cytokine receptors to transduce a proliferative signal. Ba/F3 cells are absolutely dependent on IL-3 for proliferation, but do not proliferate in response to IL-11, LIF or IL-6. It was determined, therefore, whether expression of Nr1, gp130 and the LIF receptor broadened the spectrum of cytokines to which these cells could respond. While none of the cell lines examined could proliferate in response to IL-6 alone, each cell line that expressed gp130, irrespective of whether or not other receptors were co-expressed, proliferated in response to a combination of IL-6 and the soluble IL-6 receptor α-chain (Figure 7). Proliferation in response to LIF required coexpression of the LIF receptor and gp130 (Figure 7), however, these cells were unable to proliferate in response to IL-11. Likewise, Ba/F3 cells expressing Nr1 alone or Nr1 and the LIF receptor were incapable of responding to IL-11 (Figure 7). Response to IL-11 required

coexpression of both Nr1 and gp130 (Figure 7). Half-maximal proliferation of these cells occurred at an IL-11 concentration of between 20 and 100 pg/ml. Expression of the LIF receptor, in addition to Nr1 and gp130, did not alter this response (Figure 7).

EXAMPLE 14

CLONING OF THE HUMAN IL-11rα

In order to determine the feasibility of cloning the human IL-11rα based on homology with the murine receptor, analysis of murine and human genomic DNA was carried out using a murine IL-11rα cDNA fragment as a probe (for method see Example 13). Figure 10A shows a specific band of 14 kb in human DNA, compared with 4.8 kb in the murine DNA, when examined under conditions of high hybridisation stringency (0.2 X SSC, at 65°C).

The same murine probe (445 bp *Sph I/Sac* I fragment) was then used to screen approximately 10^6 plaques from five human cDNA libraries. These included two adult bone marrow libraries (27; Clontech Cat. no. HL1058a) and libraries from the human placenta (Clontech Cat. no. HL1008b), liver (Clontech Cat. no. HL1001a) and a hepatoma cell line (Clontech Cat. no. HL1015b). Positive plaques were isolated and purified by successive rounds of hybridisation-screening (for method see Example 17). Approximately 30 positive clones were obtained from each of the adult bone marrow libraries and the placental library. No positive clones were identified from the liver or hepatoma libraries despite the murine receptor being isolated from this tissue (see previous Examples). The positive plaques were also examined using a PCR-based strategy; plaque eluates were used as templates in a PCR reaction primed with an antisense oligonucleotide encoding the murine WSXWS motif and an appropriate oligonucleotide primer derived from the vector sequence in the region adjacent to the cloning site. Three clones from a bone marrow library were initially chosen for detailed characterisation. Southern analysis using a restriction fragment from the human cDNA identified equivalent bands to those detected using the murine IL-11rα, thus confirming the identity of the human cDNA (Fig. 10B). The nucleotide sequence of the insert from each of these clones (#9.1, #4.3, #8.2), was determined in both directions. The insert from clone #9.1 was used to generate a probe to re-screen the bone marrow cDNA

library and resulted in the identification of another unique clone (#17.1, Fig. 11). The nucleotide sequence of this clone was also determined in both directions.

EXAMPLE 15

SEQUENCE ANALYSIS OF THE HUMAN IL-11rα

As depicted in Fig 11, clones #9.1, and #4.3 were incomplete while clones #8.2 and #17.1 encompassed the entire coding region. Clones #8.2 and #17.1 contained a 287 bp intronic sequence and clones #4.3 and #8.2 contained a 254 bp intronic sequence. These sequences were confirmed as introns by analysis of genomic DNA clones, exhibited typical splice donor-acceptor sequences and were attributed to incomplete splicing of mRNA. Figure 8 shows the composite nucleotide sequence determined from the four IL-11rα cDNA clones. The sequence included 127 bp of 5′ untranslated region (UTR) that was represented in 3 clones, and a 3′ UTR with a polyadenylation signal and poly A tail. There was an open reading frame of 1269 bp which was predicted to encode a protein of 432 amino acids (a.a.). The predicted protein had a potential hydrophobic leader sequence (1-23 a.a.), extracellular region (24-366 a.a.), transmembrane domain (367-392 a.a.) and a cytoplasmic tail (393-423 a.a.). The extracellular domain contained two possible sites of N-linked glycosylation (Fig. 8). As with the murine IL-11rα (see previous Examples) and in common with other cytokine receptors (15;28), the human IL-11rα exhibited an immunoglobulin-like domain and an hemopoietin domain (D200, Fig. 8) in the extracellular region. The latter was composed of two subdomains of 100 a.a. (SD100, Fig. 8) and included proline residues preceding each subdomain, four conserved cysteine residues, a series of polar and hydrophobic residues an the WSXWS motif. The variable amino acid "S" was identified as theonine in the human receptor compared to alanine in the murine equivalent (see previous Examples).

Several differences were noted between clones isolated from the same library. A nucleotide substitution in clone #4.3 (G↔C at 944 bp, Fig. 8) resulted in a different amino acid residue (E↔Q at 273 a.a., Fig. 8). Clone #4.3 and #17.1 differed from clone #8.2 by a nucleotide substitution (G↔A at 1135 bp, Fig. 8) in the coding region with no consequent change in protein. Also, clones #17.1 and #8.2 differed in the 3′ UTR by a single substitution (A↔G at 1658 bp, Fig. 8). These differences were

interpreted as representing polymorphisms.

Comparison of the sequences of the murine and human IL-11rα chains showed a high degree of homology (Fig. 12). There was overall 85% identity at the nucleic acid level and 84% at the protein level. The homology was more evident in the extracellular and transmembrane regions and less so in the cytoplasmic tail where the human receptor was 8 amino acids shorter than the murine equivalent. Neither protein contained an identifiable tyrosine kinase like domain.

EXAMPLE 16

EXPRESSION OF THE HUMAN IL-11 RECEPTOR A CHAIN RESULTS IN SPECIFIC BINDING OF HUMAN IL-11 AND PERMITS IL-11 SIGNALLING

The murine myeloid leukemic cell line M1 (29) constitutively expresses murine gp 130 the signalling molecule for LIF, IL-6, OSM and IL-11 receptors. In response to LIF, OSM and IL-6, colonies of parental M1 cells in semisolid agar become dispersed as cells differentiate into macrophages and acquire the ability to migrate through agar. In addition, there is suppression of clonogenicity leading to reduced colony numbers. M1 cells manipulated to express the murine IL-11rα displayed specific binding of IL-11 and differentiated in response to IL-11 (see previous Examples). The human IL-11rα was expressed in murine M1 cells using the mammalian expression vector pEFBOS (30; Example 15). Binding studies using ^{125}I-labelled human IL-11 were carried out to test whether IL-11 specifically bound to the these cells (see Example 15 for methods). As shown in Table 3, M1 cells manipuated to express the human IL-11rα (pools #1 - #4) demonstrated significant specific binding of human IL-11. The positive control cells, M1 cells and Ba/F3 cells expressing the murine IL-11rα and murine gp130 (see previous Examples) also showed high level binding. As expected, the parental M1 cells exhibited no detectable specific binding of IL-11. Scatchard analysis of saturation isotherms of IL-11 binding to M1 cells that expressed human IL-11rα confirmed high-affinity binding (Fig. 13). The apparent equilibrium dissociation constant (K_d) was estimated to be 250 pM. These cells expressed an average 3190 receptors at their surface. This result was comparable to M1 cells expressing murine IL-11rα (K_d=275 pM, and 4815 receptors/cell) and was attributed to an interaction of the human IL-11rα with murine

gp130.

Table 4 summarises the results of agar culture experiments of M1 cells that expressed human IL-11rα and shows their response to LIF and IL-11. As described above, M1 cells expressing the murine IL-11rα displayed clonal suppression and macrophage differentiation in response to IL-11. In contrast, the central parental M1 cells did not respond to IL-11. The four pools of M1 cells manipulated to express the human IL-11rα when treated with IL-11, showed marked suppression of clonogenicity (Table 4). In addition, the few colonies that grew in IL-11 displayed a differentiated phenotype. All cells lines showed the expected response to LIF.

M1 cells expressing human IL-11rα and control cells were also examined in suspension cultures to assess macrophage differentiation in response to IL-11 and LIF (31; 32). Macrophage morphology was assessed after five days in culture. As shown in Fig. 13, the majority of the cells displayed a macrophage phenotype following stimulation with IL-11. Similar results were observed with M1 cells expressing the murine IL-11rα, while parental M1 cells did not respond to IL-11. Thus, these experiments documented the ability of the isolated human cDNA to encode a functional receptor protein and demonstrated that co-operation between the human IL-11rα and murine gp130 was sufficient for signal transduction.

To directly address the requirement of gp130 to human IL-11 receptor signalling, murine Ba/F3 cells were examined. These cells are totally dependent on IL-3 for survival and do not constitutively express gp130. Ba/F3 cells were manipulated to express human IL-11rα and expanded based on the expression of the co-electroporated puromycin-resistance gene. Three clonal cell lines were established. These were confirmed to express human IL-11rα as assessed by binding of radio-labelled human IL-11, albeit at low level (106; 97; 116; mean specific counts bound per 10^6 cells versus undetectable binding for parental Ba/F3 cells). As shown in Fig. 14 these cells were unresponsive to IL-11. The human gp130 molecule was then expressed in each of these clonal cell lines:cells then proliferated in response to IL-11 (Fig. 14). This result confirmed the expression of the human IL-11rα in Ba/F3 cells and the requirement for gp130 for

proliferation. Parental Ba/F3 cells used as control did not respond to IL-11 and, as expected, all cells proliferated in response to murine IL-3.

EXAMPLE 17

HUMAN LIBRARY SCREENING

The following human cDNA libraries were screened using the above mentioned murine probe:two bone marrow libraries (27; Clontech Cat. no. HL1058a), a placental library (Clontech Cat. no. HL1008b), a liver library (Clontech Cat. no. HL1001a), and a hepatoma cell library (Clontech Cat. no. HL1015b). Approximately 10^6 plaques from each library were lifted onto nitrocellulose membranes and fixed by incubating at 80°C for 2 hr. under vacuum. The filters were pre-hybridised for 1 hr. and then hybridised at 65°C for 16 hr. in a solution containing 2 X SSC, 2 mg/ml bovine serum albumin, 2 mg/ml ficoll, 2 mg/ml polyvinylpyrrolidine, 100 μM ATP, 50 μg/ml tRNA, 2 mM sodium pyrophosphate, 2 mg/ml salmon sperm DNA, 200 μg/ml of sodium azide and 1% w/v SDS. The Filters were finally washed for 30 mins. at 65°C with 0.2 X SSC, 0.1% SDS. Positive plaques on duplicate filters were isolated and purified by further rounds of hybridisation screening.

Human clone #91. (Fig. 11) was also labelled and used to probe one human bone marrow cDNA library. This resulted in clone #17.1.

An amount of 15μg of human genomic DNA (obtained from peripheral blood leucocytes) and murine genomic DNA (obtained from the FDCP-1 cell line) was digested to completion with the restriction enzyme Hind III (Boehringer Mannheim, Germany). DNA fragments were separated on an 0.8% w/v agarose gel and transferred with 0.4 M NaOH on to nylon membrane (Gene Screen Plus, Biotechnology Systems, NEN Research Products).

A 445 bp *Sph* I/*Sac* I restriction enzyme digest fragment from the murine IL-11rα clone 30.1 (see earlier Examples) and a 560 bp *Pst* I/*Sba* I restriction digest fragment from the human cDNA clone #17.1 were used as probes. An amount of 100 ng of DNA was labelled using a random decanucleotide labelling kit (Braesatec, Adelaide, S.A.,

Australia). The incorporated [^{32}P] ATP was separated from unincorporated label using a NICK column (Pharmacia, Uppsala, Sweden). The membrane was prehybridised and hybridised at 65°C overnight in the buffer recommended by the manufacturer. The membrane was finally washed in 0.1% w/v SDS, 0.2 X SSC (30 mM sodium chloride, 3 mM tri-sodium citrate) for 30 min. at 65°C.

EXAMPLE 18

ANALYSIS OF HUMAN IL-11rα POSITIVE PLAQUES

Positive plaques isolated using the murine probe were further screened by a PCR-based strategy. Eluate from pure plaques (5 µl) was used as a template in a 50 µl volume PCR reaction using 2.5 U Taq polymerase (Boehringer Mannheim, Germany), the supplied buffer, 200 µM of each dNTP. The reaction was primed with 250 ng of an anti-sense oligonucleotide primer corresponding to WSXWS motif 5'-[(G/A)CTCCA(N)GC(G/A)CTCAA-3'] (SEQ ID NO. 23) and an appropriate vector oligonucleotide primer that flanked the cloned cDNA:T3 and T7 promoter primers for pBluescript plasmid, and the appropriate γgt10 and γgt11 forward and reverse primers. Control reactions that lacked the template were also performed. Three plaques (#91., #4.3, #8.2 isolated from a bone marrow library) were selected. The cDNA were sequenced on both strands using the dideoxy-termination method (18) and the Pharmacia T7 polymerase sequencing kit (Pharmacia, Uppsala, Sweden).

EXAMPLE 19

HUMAN IL-11rα EXPRESSION CONSTRUCTS AND BIOLOGICAL ASSAYS

A composite cDNA construct including the entire coding region and the polyadenylation signal but excluding the intronic sequences was made by ligating restriction enzyme digest fragments from #9.1 (*Eco* RI/*Pst* I fragment) and #17.1 (*Pst* I/*Eco* RI fragment). The construct was cloned into the *Bst* XI site of pEF-BOS (30) using *Bst* XI adaptors (Invitrogen, San Diego, CA, USA). It was linearized with *Aat* II prior to electroporation into M1 and Ba/F3 cells. pPGKpuropA and pPGKneopA are pBluescript derivatives containing the cDNA encoding puromycin transferase and neomycin transferase and were co-electroporated into cells and used as a selection markers. Human gp130 cloned into pEF-BOS was electroporated in BaF3 cells manipulated to express the human IL-11rα.

M1 cells (29) were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% v/v Fetal Calf Serum (FCS) in 10% v/v CO_2 at 37°C. Ba/F3 cells (33) were grown in RPMI-1640 medium containing 10% v/v FCS and WEHI-3B D$^-$-conditioned media as a source of IL-3 (34). M1 and Ba/F3 cells stably expressing the human IL-11rα construct were generated by electroporation as described above. Cells were co-electroporated with pPGKPuropA. Clones of Ba/F3 expressing human IL-11rα were expanded with puromycin antibiotic selection and human gp130 was introduced with pPGKneopA. These cells were expanded in G418.

For biological assays, M1 cells (300 per ml) were cultured in DMEM, 20% v/v FCS, 0.3% w/v agar and with human IL-11 (1000 U/ml) or murine LIF (1000 U/ml) or normal saline. Cultures were incubated in humidified air with 10% v/v CO_2 at 37°C. After 7 days colonies were counted and differentiation was assessed using standard criteria (35). In suspension cultures $1.5x10^4$ M1 cells were cultured in 1.5 ml of DMEM containing 10% v/v FCS and with or without IL-11 (1000 U/ml) or LIF (1000 U/ml) and incubated as above. Differentiation was determined by morphological examination of May-Grunwald Giemsa stained cells: a minimum of 200 cells was examined.

The proliferation of Ba/F3 cells was measured in a microwell assay as described above. Briefly, 200 cells/well were incubated in 15 µl of media containing the following stimuli: normal saline, murine interleukin-3 (IL-3) at final concentration 1000 units/ml and series dilutions of human IL-11. Viable cells were counted after 48 hours.

Iodination of IL-11 using the Bolton-Hunter reagent and binding studies with M1 and Ba/F3 cells were performed as previously described above.

EXAMPLE 20

SOURCE OF CYTOKINES

Murine IL-3 and human IL-11 was purchased from Peprotech (Rocky Hill, NJ, USA) and murine LIF and AMRAD Pty. Ltd. (Melbourne, Australia). Human IL-11 used in ligand binding studies was obtained by expression in COS-M6 cells. Briefly, a cDNA encoding the mature protein for human IL-11 was obtained by polymerase chain reaction

from cDNA derived from a human stromal cell line 197/17 (36). The human IL-11 mature coding region was inserted into pEF/IL3SIG/FLAG which is a pEF-BOS (30) derived expression vector containing sequences encoding the murine IL-3 signal sequence followed by the FLAG sequence (Eastman Kodak, CT, USA), and then expressed in COS-M6 cells resulting in the secretion of a biologically active human IL-11 protein with a N-terminal flag. The N-terminal flag human IL-11 was purified by affinity chromatography on an anti-FLAG M2 monoclonal antibody column (Eastman Kodak, CT, USA) as recommended by the manufacturer with peptide elution followed by gel filtration chromatography on Superdex 75 (Pharmacia, Uppsala, Sweden). The purified protein gave a single band of MW 25,000 on SDS polyacrylamide gels.

EXAMPLE 21

Since antibodies to the IL-11 receptor α chain were not available to monitor expression, constructs were engineered to express a soluble version of the murine IL-11 receptor α chain with an N-terminal FLAG epitope (International Biotechnologies/Eastman Kodak, CT, USA). First a derivative of the mammalian expression vector pEF-BOS was generated so that it contained DNA encoding the signal sequence of murine IL-3 (MVLASSTTSIHTMLLLLLMLFHLGLQASIS) and the FLAG epitope (DYKDDDDK), followed by a unique *Xba* I cloning site. This vector was named pEF/IL3SIG/FLAG.

PCR was performed using to amplify DNA fragments encoding the extracellular domain without the transmembrane or cytoplasmic regions (S24 to Q367). The primers used were:

5'-ATCTTCTAGATCCCCCTGCCCCCAAGCT-3' (SEQ ID NO: 24)

5'ACTTTCTAGATTATTGCTCCAAGGGGTCCCTGTG-3' (SEQ ID NO: 25)

The soluble murine IL-11 receptor α chain PCR product was digested with *Xba* I and cloned, in frame, into the XbaI site of pEF/IL3SIG/FLAG to yield pEF-sIL-11rα.

In order to confirm soluble murine IL-11 receptor α chain could be produced using the expression vectors pEF-SIL-11rα, COS cells were transiently transfected with these constructs. Briefly, COS cells from a confluent 175 cm^2 tissue culture flask were resuspended in PBS and electroporated (BioRad Gene pulser; 500 μF, 300 V) with 20

μg of uncut pEF-sIL-11rα in a 0.4 cm cuvette (BioRad). After 2 to 3 days at 37°C in a fully humidified incubator containing 10% v/v CO_2 in air cells were used for analyses of protein expression. Conditioned medium was collected by centrifugation and stored sterile at 4°C.

Medium was then chromatographed on an anti-FLAG antibody affinity column (International Biotechnologies/Eastman Kodak, CT, USA). Proteins that failed to bind to the column were washed through with PBS containing, while those proteins the murine IL-11 receptor α chain proteins which bound to the column was eluted with 8 ml of ug/ml FLAG peptide. The purified soluble murine IL-11 receptor α chain was electrophoresed on a SDS-polyacrylamide gel, which was stained with silver to reveal the presence of a major band with an apparent molecular weight of approximately 40,000 similar to the predicted size of the soluble murine IL-11 receptor α chain.

The purified soluble murine IL-11 receptor α chain was tested for its ability to stimulate the differentiation of M1 cells in the presence or absence of IL-11. IL-11 and the soluble murine IL-11 receptor α chain were unable to stimulate M1 differentiation alone, however, when combined, differentiation was observed in both liquid and semi-solid culture. These results demonstrate that soluble murine IL-11 receptor α chain may act as an agonist, allowing IL-11 to exert effects on cells expressing gp130 in the absence of membrane bound IL-11 receptor α chain. In this way soluble IL-11 receptor α chain is similar to soluble IL-6 receptor α chain.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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